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## Encapsulation of fish oil and essential fatty acids by spray drying

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Fish oil and essential fatty acids are considered significant in functional foods. Polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (20 : 5) and docosahexaenoic acid (22 : 6) in fish oil are prone to oxidative damage. The encapsulation of fish oil by spray drying is a useful method to stabilize fish oil by reducing contact with oxygen. In this review, kinetic analyses of oxidation of fish oil and fatty acids in bulk and emulsion systems and encapsulated fish and krill oils in spray-dried powders were reviewed. Oxidation kinetics of PUFAs could be expressed with the autoxidation equation using the fraction of unoxidized PUFA. The surface-oil ratio could be correlated with the equation  $1 - (1 - 2(de/dp))^3$  using the ratio of oil-droplet diameter,  $de$ , to powder diameter,  $dp$ . The morphology of the spray-dried powder containing vacuoles affected the surface-oil ratio. The activation energy ( $E_a$ ) could be correlated with the reconstituted oil droplet diameter of squalene powders. In the estimation of fish oil in spray-dried powder, this review showed that the model of deterioration kinetics of fish oil in the powder is very useful.

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### Sustainability spotlight

The technological development of “encapsulation” to form functional foods such as fish oil with a high content of polyunsaturated fatty acids (PUFAs) in a stable form is expected to promote the No. 3 purpose, “Ensure healthy lives and promote well-being for the super-aging society,” in SDGs. It is very important to realize “healthy lives for all at all ages” by using stable functional foods. This review paper summarized especially the degradation kinetics of PUFAs in liquids and the emulsified spray-dried powder. We believe that it will be very useful for advancing the basic technology for safe intake of functional food in order to maintain the health of elderly people.

## Introduction

In recent years, the population of elderly people has been increasing rapidly. To this end, the food industry has developed functional foods to regulate the physiological characteristics of people, such as blood fluidity, blood pressure, and intestinal activity. Popular functional foods include dietary fibres, lactic acid bacteria, and n-3 (omega-3) polyunsaturated fatty acids (PUFAs), which are considered essential fatty acids. n-3 PUFAs, such as **EPA** (20 : 5) and **DHA** (22 : 6), are useful for preventing disorders such as cardiovascular disease,<sup>1</sup> inflammation,<sup>2</sup> cancer,<sup>3</sup> and hypertension.<sup>4</sup> As fatty acids, PUFAs play an important role in human nutrition and disease prevention. Many studies have reported that a high intake of n-3 fatty acids results in a low mortality of coronary artery disease.<sup>5</sup> A review on **EPA** and **DHA** intake and death from coronary artery diseases

has been reported previously.<sup>6</sup> Numerous reviews and meta-analyses have examined the evidence concerning the cardiovascular effects of n-3 PUFAs.<sup>7,8</sup> The global active pharmaceutical ingredient market was valued at USD 187.76 billion in 2020 and is expected to grow at a compound annual growth rate of 6.6% from 2021 to 2028 (Active Pharmaceutical Ingredients Market Report, 2021).

The main sources of n-3 fatty acids are fish oils, krill oil, and marine oils; they can also be found in plant products, such as flaxseeds and nuts. Fish oil is a good source of long-chain n-3 PUFAs. **EPA** and **DHA** have double bond structures that include 5 and 6 double bonds in a carbon chain of 20 and 22, respectively, with n-3 indicating the position of the first double bond in the acyl chain, as shown in Fig. 1.

Fish oils, which are an important source of n-3 fatty acids, are suitable for direct incorporation into foods; however, it is difficult to protect them from oxidation owing to their high degree of unsaturation. Anchovy and sardine oils are major sources of **EPA** and **DHA**, and currently, these two species account for 80% of n-3 products in the market.<sup>9</sup> These oils were reported to have the highest concentrations of **EPA** and **DHA** (over 18% and 12%, respectively).<sup>10</sup> Durmus (2018)<sup>11</sup> reported that the fatty acid composition of seafood species included

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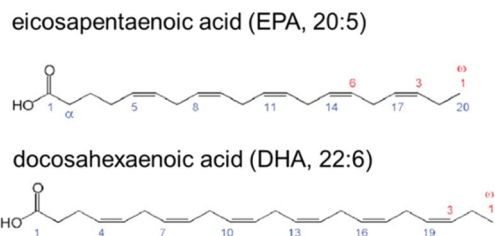


Fig. 1 Chemical structures of EPA and DHA.

10.69–39.57% PUFAs, 1.72–10.73% **EPA**, and 4.07–31.44% **DHA**. All seafood species had high **EPA** and **DHA** levels and a significantly higher n-3 PUFA content than the n-6 PUFA content. As **EPA** and **DHA** are important functional compounds for human health, the Japanese fisheries company, Maruha Nichiro Corporation, produces high **DHA**-content oil. This oil is produced by the enzymatic hydrolysis of raw tuna oil using lipase to obtain an undecomposed reaction product containing concentrated free fatty acids and **DHA**. Fatty acids are removed from this oil mixture to obtain a triacylglycerol fraction. This product is decolourised with active soil and deodorised by steam distillation to produce **DHA**-45, which is a triacylglycerol comprising 45% **DHA** and 4.6% **EPA**.<sup>12</sup> Dahiya *et al.* (2023)<sup>13</sup> reviewed the current status and future prospects of bioactive molecules delivered through sustainable encapsulation techniques for food fortification. Eratte *et al.* (2018)<sup>14</sup> reviewed the microencapsulation of n-3 oil through complex coacervation.

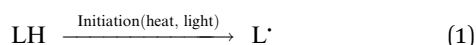
However, there are very few papers on the oxidation kinetics of PUFAs in spray-dried powders.

These functional food materials such as fish oil, krill oil or PUFAs are very prone to oxidation. In this paper, kinetic analyses of oxidation of fish oil and fatty acids in bulk and encapsulated fish and krill oils in spray-dried powders are reviewed.

## Autoxidation kinetics of PUFAs and their esters

PUFAs, such as **EPA** and **DHA**, are sensitive to oxidative damage. Lipid oxidation in foods was reported by Simic *et al.* (1996).<sup>15</sup> Hammond and White (2011)<sup>16</sup> reviewed a brief history of studies on lipid oxidation. Bolland and Gee (1946)<sup>17</sup> presented the basis of the basic autoxidation scheme, as shown in eqn (1)–(3); they presented the kinetics of the oxidative processes during lipid oxidation and oxidation mechanism, including initiation, propagation, and termination. (i) The initiation step comprises the monomolecular step of hydroperoxide formation and peroxy radical scavenging by antioxidants; (ii) the propagation step includes autocatalytic, monomolecular, and bimolecular or branching reactions; and (iii) the termination stage is primarily characterised by hydroperoxide decomposition and increased formation of secondary oxidation products.

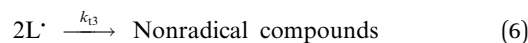
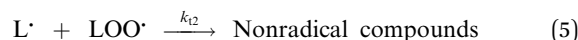
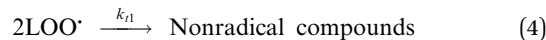
Initiation:



Propagation:



Termination:



Lipid oxidation comprises complex reactions in which the aforementioned three processes are intricately intertwined; hence, it is difficult to analyse the rate of lipid oxidation.

The autoxidation of PUFAs or their esters is a complicated process, and the aforementioned kinetics are typically considered for describing the individual steps. Özilgen and Özilgen (1990)<sup>18</sup> proposed a kinetic model to describe the entire oxidation process of lipids. Adachi, Ishiguro, & Matsuno (1995)<sup>19</sup> proposed an autocatalytic-type equation for describing the entire oxidation process of n-6 PUFAs and the first half of the oxidation process of n-3 PUFAs based on the kinetic equation proposed by Bolland and Gee (1946)<sup>17</sup> for the propagation step of lipid oxidation. The oxidation rate of PUFA was assumed to be proportional to the product of the concentrations of unoxidised and oxidised PUFAs.

$$dC_{\text{LH}}/dt = -k' C_{\text{LH}}(C_{\text{LH},t} - C_{\text{LH}}), \quad (7)$$

where  $C_{\text{LH}}$  is the concentration of unoxidised PUFA,  $C_{\text{LH},t}$  is the total concentration of unoxidised and oxidised PUFAs,  $t$  is the time, and  $k'$  is the intrinsic rate constant. Eqn (7) can also be expressed as follows:

$$dY/dt = -kY(1 - Y) \quad (8)$$

where  $Y(=C_{\text{LH}}/C_{\text{LH},t})$  is the fraction of unoxidised PUFA, and  $k$  is the rate constant defined as  $k' C_{\text{LH},t}$ . The integration of eqn (8) under the condition  $Y=Y_0$  at  $t=0$  yields eqn (9) or eqn (10):

$$\ln \frac{1 - Y}{Y} = kt + \ln \frac{1 - Y_0}{Y_0} \quad (9)$$

$$Y = \frac{1}{1 + [(1 - Y_0)/Y_0]e^{kt}} \quad (10)$$

The parameter  $Y_0$ , which reflects the initial state of the PUFA to be oxidised, is close, but not equal to 1. Eqn (8) describes the entire oxidation process of PUFAs and their esters. Fig. 2 shows the autoxidation processes of ethyl arachidonate (EtARA) and ethyl docosahexaenoate (EtDHA) at 50 °C and 75% relative humidity. The inset of Fig. 2 shows the applicability of eqn (9) to the oxidation process. The equation was applicable to the entire oxidation process of EtARA and the first half of the oxidation



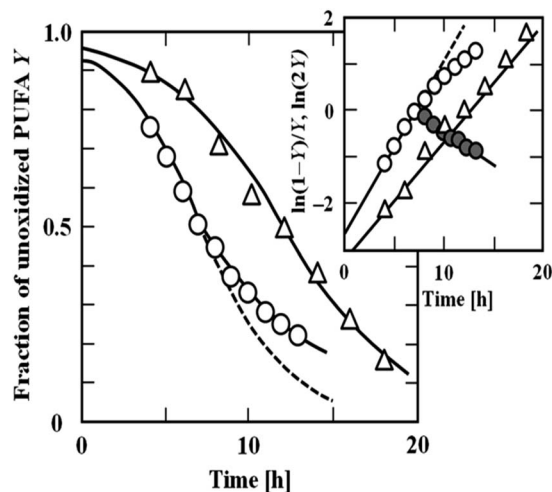


Fig. 2 Changes in the fraction of unoxidized ( $\Delta$ ) ethyl arachidonate (EtARA) and ( $\circ$ ) ethyl docosahexaenoate (EtDHA) during autoxidation at 50 °C and 75% relative humidity. Inset: applicability of eqn (9) to the autoxidation processes of EtARA and EtDHA. The closed symbol ( $\bullet$ ) indicates that the latter half of the oxidation of EtDHA obeyed the first-order kinetics (Adachi *et al.*, 1995).<sup>19</sup> "Reproduced from ref. 19 with permission from JAOCS., copyright 1995".

process of EtDHA. The latter half of the oxidation process of EtDHA cannot be described by eqn (8), but it can be simply expressed by first-order kinetics. The kinetic equations were also applicable to the autoxidation processes of other PUFAs and their acylglycerols.<sup>20</sup>

PUFAs increase in weight as they are oxidised, and the autoxidation of PUFAs is exothermic. The autoxidation processes of the ethyl esters of PUFAs were monitored using thermogravimetry, calorimetry, and gas chromatography to investigate the stoichiometric coefficient of the PUFA and oxygen.<sup>21</sup> The change in weight ( $\Delta w$ ) during the oxidation is related to the fraction of the unoxidised PUFA ( $Y$ ), as shown in eqn (11).

$$\frac{d(\Delta w/w_0)}{dY} = \frac{M_X}{2M_F} \frac{dn_X}{dn_F} \quad (11)$$

where  $w_0$  is the initial weight of PUFA,  $M$  is the molecular mass,  $n$  is the amount of substance (mol), and the abbreviations X and F denote oxygen and PUFA, respectively. The term  $dn_X/dn_F$  denotes the stoichiometric coefficient. Fig. 3 shows the weight gains of EtARA and EtDHA during oxidation at 50 °C. The solid line represents the relationship between relative weight gain and unoxidized PUFA, when the coefficient is 1. The plotted points for EtARA are scattered along this line, indicating that the coefficient is independent of the fraction of unoxidised EtARA throughout the entire autoxidation process. The plotted points for EtDHA in the range of  $Y > 0.5$  lay along the line, but they deviated from the line when  $Y$  was less than 0.5. The coefficient calculated from the slope changed from 1 to approximately 5 with a decreasing  $Y$  value.

The oxidation behaviours of unsaturated fatty acids were represented by the autocatalytic reaction using  $Y$ , except when  $Y$  of n-3 unsaturated fatty acids is above 0.5. This formula could

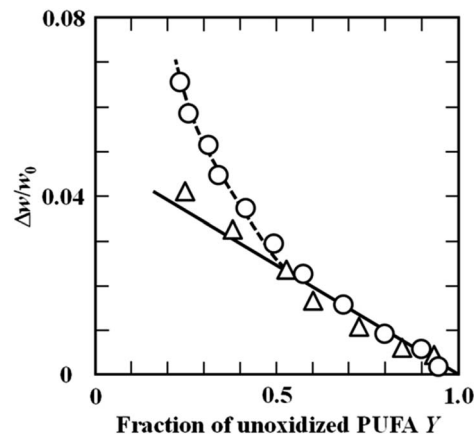


Fig. 3 Relationships between the relative weight gain  $\Delta w/w_0$  and fraction of the unoxidised substrate ( $Y$ ) for ( $\Delta$ ) ethyl EtARA and ( $\circ$ ) EtDHA at 50 °C (Adachi *et al.*, 1995).<sup>21</sup> "Reproduced from ref. 21 with permission from *Food Sci Technol Int*, copyright 1995".

also be applied to powder using the model in which the free energy of activation for the rate constant is assumed to obey a Gaussian distribution (Ishido *et al.*, 2002).<sup>22</sup>

## Spray drying as a method to encapsulate fish oil and essential fatty acids

Spray drying is a technique to obtain powders with a submicron-to-micron size range containing oil-droplets such as functional food oil from the emulsion or slurry solution. Spray drying could form functional food powders, pharmaceuticals, and food powders such as soup or juice. Fig. 4 shows the encapsulation processes of fish oil by spray drying. The first important operation factor is the inlet air temperature, which affects the moisture content in the powder, powder recovery efficiency, and powder morphology. The second important operation factor in spray drying is the rotation speed of the rotary atomizer or the gas pressure of the one- or two-fluid nozzle. Atomizer conditions affect the powder particle size and also the oil-droplet size.

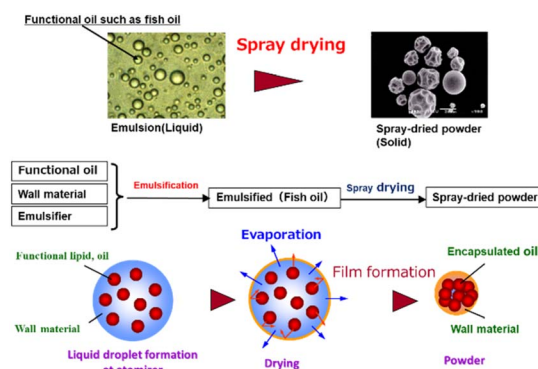


Fig. 4 Encapsulation processes of fish oil by spray drying.



High shear-stress at the atomizer affects the aggregation behaviour of the oil-droplets in the emulsion.

### Wall materials

The selection of wall materials is an important step in forming a stable emulsified fish-oil powder. Wall materials play a crucial role in stabilizing fish oil and form good flowable powder. To get oxidative stable fish oil, the wall material should have low oxygen diffusivity and a dense coating film as an oxygen-transfer barrier. In order to obtain a dense film, a low-molecular-weight sugar might be preferable to form a dry-film quickly and easily during spray drying. However, low-molecular-weight sugars have low glass transition temperature and a low powder recovery efficiency in spray drying. When sucrose was used as the wall material, the starch coating process was attached to a spray dryer.<sup>23</sup> Maltodextrin as an additive to the sugar wall material is used to control the glass transition temperature.

Xiao *et al.* (2022)<sup>24</sup> reviewed maltodextrin as a wall material for microcapsules. The dextrose equivalent (DE) of MD affected the morphology of spray-dried powder. Siemons *et al.* (2020)<sup>25</sup> showed DE of MD determined particle morphology during single sessile drying.

Low-molecular-weight sugar and maltodextrin do not have antioxidant ability. Proteins as wall materials could not form dense films, but whey protein or caseinates have antioxidant and also emulsifying abilities. The selection of wall materials should be determined by whether or not a stable fish oil powder can be obtained.

### Preparation of emulsions

An emulsification process is used to facilitate the powder formation of fish oil; in this process, fish oil is mixed with a solution comprising an emulsifier and a wall material, followed by a spray drying process, in which the emulsified solution is atomised into droplets in hot air and spray dried, as shown in Fig. 4.

The emulsification process is aimed at reducing the oil droplet size and producing a stable emulsion solution. The emulsification process is crucial because it affects the powder properties significantly. In particular, the oil droplet size in an emulsion is an important factor that determines the proportion of fish oil in the spray-dried powder and the proportion of unencapsulated fish oil represented by the surface-oil ratio. Therefore, the selection of an emulsifier is important for the formation of fish oil powder. In the food industry, emulsified functional oil is prepared using a high-pressure homogenizer using about 20 MPa. In the formation of emulsified fish oil, whey protein or caseinate protein at about 3–6 wt% is used as an emulsifier. But octenyl succinic acid anhydride modified starch

(OSA-starch) is often used instead of protein emulsifiers due to the increasing number of allergic patients recently.

### Drying

The liquid droplets of the emulsion in contact with hot air assist in evaporating the water from the emulsion. In this process, many factors, such as droplet size, type, solid content, inlet air temperature, and emulsion supply rate, affect the powder size and moisture content of the spray-dried powder after drying.

### The properties of encapsulated fish oil and essential fatty acids

Encapsulation efficiency (EE) is defined using the following equation:

$$EE = \frac{(\text{total weight of oil in powder} - \text{weight of surface oil in powder})}{\text{weight of total oil in powder}} \quad (12)$$

EE is affected by several parameters, such as the oil droplet diameter, solid and oil contents, and the processing conditions of spray drying. The surface-oil ratio is the most important parameter for estimating the shelf life of fish oil in spray-dried powders. The surface-oil ratio, *s*, is defined as the ratio of the amount of oil exposed on the surface of a microcapsule to the entire amount of oil within the microcapsule.

$$s = (\text{surface oil powder})/(\text{total oil in powder}) \quad (13)$$

The surface oil is related to the susceptibility of the micro-encapsulated oil to oxidation.

Jafari *et al.* (2008)<sup>26</sup> showed that nanooil droplets, with a diameter in the range of 210–280 nm, contain the lowest surface-oil ratio in encapsulating fish oil by spray drying. Ahn *et al.* (2008)<sup>27</sup> indicated that low microencapsulation efficiency of powder may lead to a higher surface-oil ratio and indicated higher lipid oxidation in the encapsulation of sunflower oil and its powder storage at 60 °C for 30 days. Carneiro *et al.* (2013)<sup>28</sup> indicated that a high encapsulation efficiency and a minimal surface-oil ratio in the spray-dried powder are essential to produce a stable powder. These studies suggest that the surface-oil ratio is crucial for evaluating the stability of encapsulated oil in spray-dried powder. Shiga *et al.* (2014)<sup>29</sup> proposed a simple method for determining the flaxseed or fish oil content in microcapsules prepared by spray drying using *N,N*-dimethylformamide (DMF). The DMF method (because DMF is used to dissolve microcapsules) reduces solvent consumption, increases the extraction yield, and enhances the extract. The extraction of surface oil from spray-dried powders by dispersion with hexane under various conditions has been reported by our group.<sup>29–32</sup>



Furthermore, hexane is known to be more effective than petroleum ether to measure the surface-oil ratio, if the samples are stored for any time duration.<sup>33</sup> Abd Ghani *et al.* (2017)<sup>34</sup> indicated that it was difficult to remove the surface oil of nano-sized oil droplets by hexane washing of spray-dried powders because of the higher stability of smaller oil droplets. Several researchers have measured the surface-oil ratio (extractable oil) of fish oil encapsulated with wall materials such as modified starch,<sup>35,36</sup> modified starch or glucose,<sup>37</sup> modified starch and whey protein,<sup>26</sup> and whey protein and sodium caseinate (NC).<sup>38</sup> Their data indicated that a higher oil droplet diameter in the spray-dried powder corresponded to a higher surface-oil ratio. Abd Ghani *et al.* (2017)<sup>32</sup> correlated the average reconstituted oil droplet diameter to the surface-oil ratio using the data reported above. No systematic investigations have been conducted on the effects of oil droplet and powder diameters on the surface-oil ratio and the encapsulated efficiency in spray-dried powders.

Fig. 5 shows an electron micrograph of the cut surface of an emulsified spray-dried fish oil powder. Many oil droplets were observed in the shell of the spray-dried powder and one large vacuole was found in the centre of the powder. Oil droplets on the surface of the vacuole can be seen in this figure.

Fig. 6 shows the locations of oil presence in spray-dried powder containing emulsified oil. While encapsulating functional oil by spray drying, the surface-oil ratio is important for evaluating the stability of functional oil in spray-dried powders. The encapsulated oil is the inside oil in closure within the wall material of the spray-dried powder. The surface oil present as adsorbed oil and contact oil exists mainly on the surface of the spray-dried powder and inside the vacuole.

### Physical properties

The physicochemical properties of the emulsified fish oil spray-dried powder are (1) the amount of fish oil content in the powder, (2) the surface-oil ratio, (3) average powder size and average oil droplet size in powder, (4) powder morphology, and (5) powder fluidity. The fish oil content of the emulsified fish oil spray-dried powder was generally 40–45 wt%, but recently, due to the increase in various operating costs, some powders had 60 wt% fish oil. In the emulsified middle-chain triacylglycerol oil case, spray-dried powder

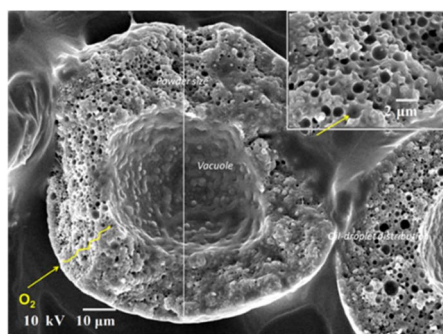


Fig. 5 Scanning electron micrograph of the cut surface of spray-dried powder containing emulsified fish oil.

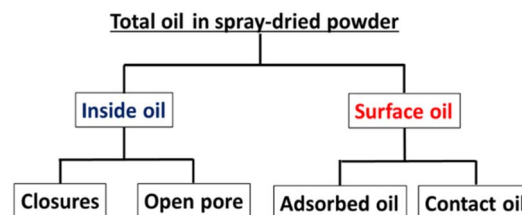


Fig. 6 Locations of oil presence in spray-dried powder containing emulsified oil.

contains almost 80 wt% in the powder. Fish oil content and the ratio of the average oil droplet diameter to the average powder diameter greatly affected the surface-oil ratio. Spray drying, which has a small encapsulation efficiency and larger surface-oil ratio in the encapsulation, is meaningless. In general, in order to produce a stable emulsified fish oil powder, it is preferable to form a powder having a small surface-oil ratio and a larger powder diameter with smaller oil droplets. A powder structure with a low percentage of voids is also important, *i.e.* solid powder forming powders with a low surface-oil ratio. Using a low-molecular-weight sugar as the wall material, a solid powder could be produced. But those sugars such as sucrose have low glass transition temperature ( $T_g$ ). When using a wall material with  $T_g$ , the powder recovery should be considered.

## Stability of encapsulated oils

### Effect of oil droplet size on the oxidation of microencapsulated PUFA

Nakazawa *et al.* (2008)<sup>39</sup> examined the effects of oil droplet size and the weight ratio of oil to the wall material on the oxidation of methyl linoleate (MeLA) microencapsulated with maltodextrin (MD) by spray drying. As shown in Fig. 7, the oxidation of MeLA was more retarded for the microcapsules prepared from emulsions with smaller oil droplets.

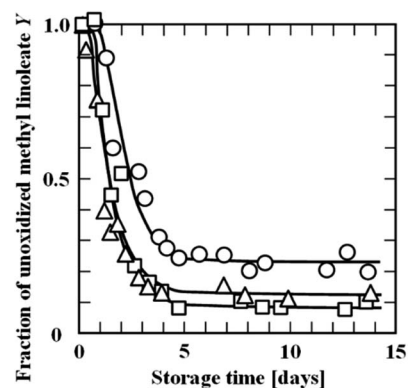


Fig. 7 Oxidation processes at 50 °C of MeLA encapsulated with MD at a weight ratio of 0.35. Microcapsules were prepared from the O/W emulsions with median oil droplet diameters of (○) 0.02, (△) 0.6, and (□) 1 μm (Nakazawa *et al.*, 2008).<sup>39</sup> "Reproduced from ref. 39 with permission from *J Oreo Sci.*, copyright 2008".



The oxidation was more suppressed for the microcapsules with a lower weight ratio of oil to the wall material. The fraction of unoxidised MeLA levelled off at  $Y_\infty$  upon prolonged storage. The dependence of  $Y_\infty$  on the weight ratio was analysed using two- and three-dimensional percolation models. These models were also applied to the oxidation processes of linoleic acid (LA) encapsulated with polysaccharides of different weight ratios by a single droplet drying method.<sup>40</sup> The effects of oil fraction and oil droplet size in microcapsules on the surface-oil ratio were examined by simulating two- and three-dimensional percolation models.<sup>41</sup> The surface-oil ratio was lower when the oil content was lower in the microcapsules, particularly with smaller oil droplets. The simulation results indicated that smaller oil droplets were more favourable for suppressing the oxidation of microencapsulated PUFAs.

The surface-oil ratios of microcapsules with different oil droplet-to-microcapsule size ratios were estimated based on the two-dimensional percolation model, assuming that the frequency function of the oil droplet-to-microcapsule size ratio can be expressed by a log-normal distribution.<sup>42</sup> The variance in the distribution of the oil droplet-to-microcapsule size ratio had no significant effect on the surface-oil ratio in the microcapsules at any oil fraction.

Oil isolated from the microcapsule surface was hard to oxidise. The two-dimensional percolation model was also applied to statistically calculate the interior oil fraction in a microcapsule, which is defined as the ratio of the volume of oil isolated from the microcapsule surface to the whole volume of the microcapsule.<sup>43</sup> As the entire oil fraction increased, the interior oil fraction first increased and then sharply decreased after reaching a maximum value. The reduction of the oil droplet size was effective in suppressing the oxidation owing to the higher interior oil fraction.

Fig. 8 shows the volume-based distribution of the reconstituted oil droplet diameter and particle diameter in the spray-

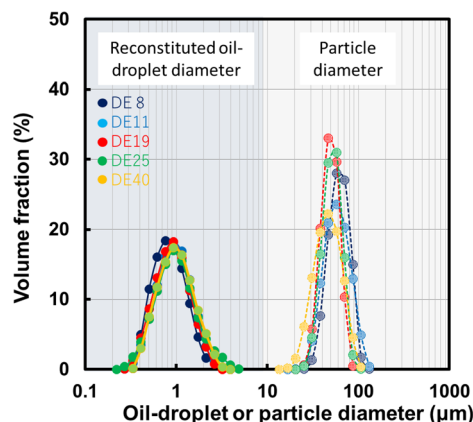


Fig. 8 Volume-based distribution of the reconstituted oil droplet diameter and particle diameter in spray-dried powder. Closed keys (solid lines) show the reconstituted oil droplet diameter and thin-colour keys (dotted lines) show the particle diameter distribution (Abd Ghani *et al.*, 2017).<sup>32</sup> "Reproduced from ref. 32 with permission from *J Chem Eng Soc. Jpn.*, copyright 2017".

dried powder. This figure shows the particle diameter and reconstituted oil droplet diameter for each DE of MD were almost the same (approximately 1  $\mu\text{m}$ ).

However with an increase in the DE of MD, these phenomena might occur because the water diffusion coefficient increases with an increase in the DE of MD. The vacuole diameter significantly depended on the DE of the MD. This vacuole affects the surface-oil ratio because the oil droplets exist at the shell of the spray-dried powder. Abd Ghani *et al.* (2017)<sup>32</sup> correlated the surface-oil ratio ( $s$ ) and the ratio of the vacuole diameter ( $d_v$ ) to the particle diameter ( $d_p$ ) and obtained a linear correlation equation,  $s = 0.42(d_v/d_p)$ .

The  $s$  can also be correlated with the ratio ( $E$ ) of the oil droplet diameter ( $d_e$ ) to the particle diameter ( $d_p$ ) as follows:

$$s = 1 - (1 - 2E)^3 \quad (14)$$

These results indicate that a higher encapsulation efficiency may be obtained with a larger particle diameter, smaller oil droplet diameter, and smaller vacuole diameter in spray-dried powder.

The effect of fish oil content added to spray-dried powder on the surface-oil ratio was investigated using  $DE = 19$  of MD.

Fig. 9 shows the relationship of the ratio ( $E$ ) for various fish oil contents in spray-dried powder. In the region where the value of  $E$  was  $10^{-2}$  or less, the surface-oil ratio was as low as 0.05, whereas in the region where  $E$  was greater than or equal to  $10^{-2}$ , the surface-oil ratio increased sharply.

#### Stability of encapsulated oil and surface oil of emulsified fish oil and squalene in spray-dried powder

Spray-dried powder with a lower surface-oil ratio can be obtained by using a nano-emulsion based on the relationship between the surface-oil ratio and oil droplet diameter, as shown in the above section. Regarding the retention of orange oil in spray-dried powders, Risch and Reineccius (1988)<sup>44</sup> reported that larger emulsion sizes had a longer shelf life. However,

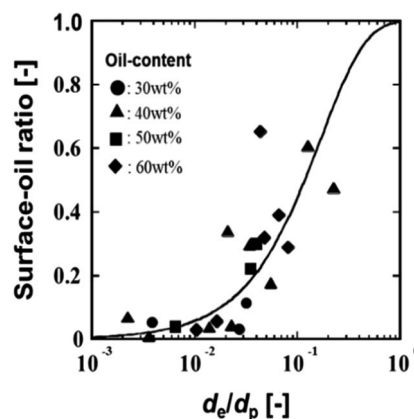


Fig. 9 Relationship of the ratio ( $E$ ) of oil droplet diameter ( $d_e$ ) to particle diameter ( $d_p$ ) and surface-oil ratios for varying fish oil content in spray-dried powder. (Abd Ghani *et al.*, 2017)<sup>32</sup> "Reproduced from ref. 32 with permission from *J Chem Eng Soc. Jpn.*, copyright 2017".



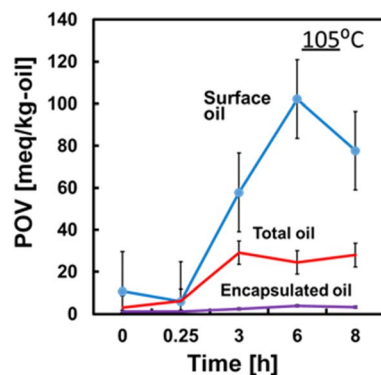


Fig. 10 Peroxidation value changes in spray-dried powder of emulsified fish oil powder during storage at 105 °C. The blue line denotes surface oil, red line denotes total oil, and purple line denotes encapsulated oil (DE of MD = 19) (Abd Ghani *et al.*, 2017)<sup>45</sup> "Reproduced from ref. 45 with permission from *Oxford Univ. Press.*, copyright 2017".

Ishido *et al.* (2002)<sup>22</sup> reported that the oxidation rate of LA contained in MD was slower as the emulsion size was smaller.

The changes in the peroxidation value (PV) were measured during the storage of spray-dried powder of emulsified fish oil at 105 °C using MD (DE19). The PV of the surface-oil was measured using fish oil in the hexane-washing solvent. The PV of the encapsulated oil was measured using a solubilised solvent of hexane-washed powder with DMF. The PV of the total oil was measured using a solubilised solvent of spray-dried powder with DMF.

Fig. 10 shows PV changes during the storage separately as the surface oil, the encapsulated oil, and total oil in spray-dried powder.<sup>45</sup> The X-axis was plotted with the number scale of sampling time. Surface oils were observed to be significantly oxidised. The PVs of surface oils increased after storage at 105 °C. The PVs of the encapsulated oils were one-order of magnitude lower than the value of surface oil. As shown in Fig. 10, the surface oil oxidised initially. Small oil droplets in spray-dried powder decrease the surface-oil ratio as shown in Fig. 9. However, smaller oil droplets had larger specific surface area, which promotes oxidation. To investigate the effect of oil droplet size on the stability of squalene (SQ) in spray-dried powder of emulsified SQ, three different types of reconstituted

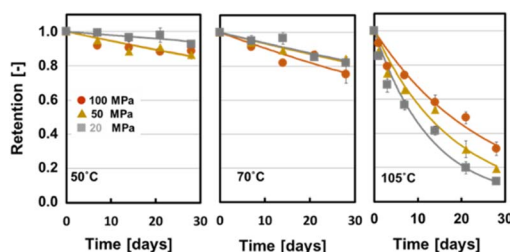


Fig. 11 Stability of squalene in spray-dried powder at three homogenisation pressures, with high-pressure homogenisation of the emulsion at 50, 70 and 105 °C. These powders contained 5 wt% NC in the solid powder. (Yoshii *et al.*, 2023)<sup>46</sup> "reproduced from ref. 46 with permission from Taylor and Francis, copyright 2023".

SQ oil droplet diameters were formed using the pressure change in a high-pressure homogeniser (20, 50 and 100 MPa). Spray-drying emulsions were prepared using 40 wt% solid content, with 40 wt% SQ in the solid, 3–8% NC, 0.4 wt% lecithin, and 56.6–51.6 wt% MD (DE = 19). SQ can be analysed with gas chromatography, and the retention of squalene in the powder can be easily measured.

Fig. 11 shows the stability of SQ in spray-dried powder. By assuming a first-order kinetics for oxidation of SQ in the powder, the first-order kinetic constants were obtained.

At 105 °C, the SQ sample with smaller oil droplets in the powder was more stable than SQ with larger oil droplets. This result suggests that the radical change in the degradation rate of SQ in the powder might be a rate-limiting process in the oil droplet in the emulsified SQ powder. At 50 °C, the powder with larger oil droplets had a lower oxidation rate because of the smaller specific surface area of the oil droplets. These results revealed that the oil droplet diameter plays a major role in the stability of the encapsulated SQ.

Fig. 12 shows the Arrhenius plot of the oxidation rate constants for 3, 5, and 8 wt% of the emulsifier NC. The regression straight lines for 100 MPa and 20 MPa homogenisations intersect with each other. The dependence of oil droplet size on SQ stability differs between high and low temperatures because of the difference in the oxidation reaction mechanism at different temperatures, as described above.

The activation energy values were estimated using these regression lines.

Fig. 13 shows the relationship between the activation energy ( $E_a$ ) [kJ mol<sup>-1</sup>] and the reconstituted oil droplet diameter ( $d_r$ ) [μm] of the SQ powders.  $E_a$  can be correlated with the following equation:

$$E_a = 43.1d_r + 22.2 \quad (15)$$

This equation indicates that the SQ oxidation kinetics can be estimated using the oil droplet diameter in the spray-dried powder. The oil droplet diameter may affect the radical transfer rate, specific surface area of the oil droplet, and/or kinetic constant of the oil volume. The frequency factors,  $k_0$  [1/day], of

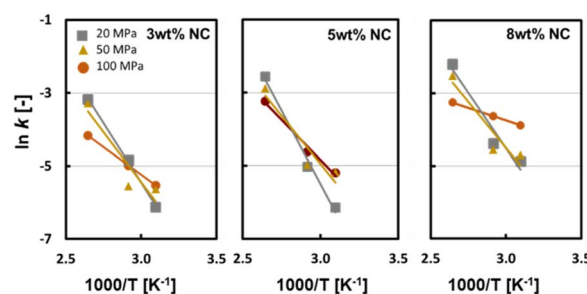


Fig. 12 Arrhenius plots of the degradation rate constant at 50, 70, and 105 °C. The powders were prepared at □: 20 MPa, △: 50 MPa, and ○: 100 MPa. (Yoshii *et al.*, 2023)<sup>46</sup> "Reproduced from ref. 46 with permission from Taylor and Francis, copyright 2023".



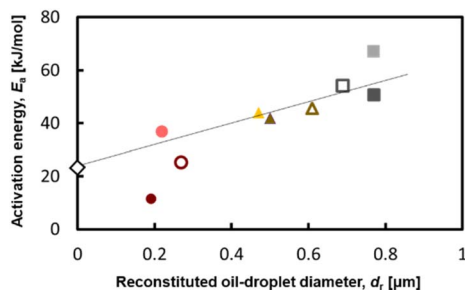


Fig. 13 Relationship between activation energy and reconstituted oil droplet diameters of the SQ powders. Powder prepared by homogenisation at  $\square$ ,  $\bullet$ ,  $\circ$ : 100 MPa,  $\Delta$ ,  $\blacktriangle$ ,  $\triangle$ : 50 MPa and  $\square$ ,  $\blacksquare$ ,  $\blacksquare$ : 20 MPa with 3 wt% NC is denoted by clear colour symbols, 5 wt% NC by light colour symbols, and 8 wt% NC by dark colour symbols. The rhombus key indicates the activation energy for the oxidation of the bulk oil of SQ. (Yoshii *et al.*, 2023)<sup>46</sup> "Reproduced from ref. 46 with permission from Taylor and Francis, copyright 2023".

SQ in these SQ systems of spray-dried powder were also correlated with the activation energies,  $E_a$  as follows:

$$\ln k_0 = 0.33E_a - 3.92 \quad (16)$$

The encapsulation process affected the stability retention of SQ in the spray-dried powder. The stability of SQ retention in the spray-dried powders, which had reconstituted oil droplet diameters of 0.69–0.77, 0.47–0.61, and 0.19–0.27  $\mu\text{m}$ , was investigated at 50, 70, and 105  $^{\circ}\text{C}$  for 28 days. Larger oil droplet diameter SQ powders had a lower degradation rate at 50 and 70  $^{\circ}\text{C}$ , and smaller oil droplet diameter powders were more stable at 105  $^{\circ}\text{C}$ .

### Stability of EPA and DHA in spray-dried powder of emulsified krill oil

Krill oil is a functional oil that is a source of n-3 PUFAs such as EPA and DHA. The global krill oil market is estimated to attain a value of USD 801.65 million by 2023. Sultana *et al.* (2021)<sup>47</sup> investigated the stability of EPA and DHA in spray-dried powders of emulsified krill oil. Krill oil was homogenised using a saponin emulsifier (Quillayanin S-100, Maruzen Pharmaceuticals Co., Ltd, Hiroshima, Japan). The spray-dried powder of emulsified krill oil was obtained using MD (DE = 19) and an antioxidant, that is, rosemary extract oil. Fig. 14 shows the EPA retention behaviour at storage temperatures of 25, 50, and 70  $^{\circ}\text{C}$ .

The solid lines in Fig. 14 are correlation lines calculated using the Avrami equation as follows:

$$R = \exp(-(kt)^n) \quad (17)$$

where  $R$  is the retention of EPA,  $k$  is the oxidation rate constant, and  $n$  is the mechanism number. The Avrami equation usually describes crystal transformation kinetics. Yoshii *et al.* (2023)<sup>48</sup> resummared the reaction mechanism with mass transfer in the powder. Matsuno and Adachi (2023)<sup>49</sup> reviewed a kinetic model in which the free energy of

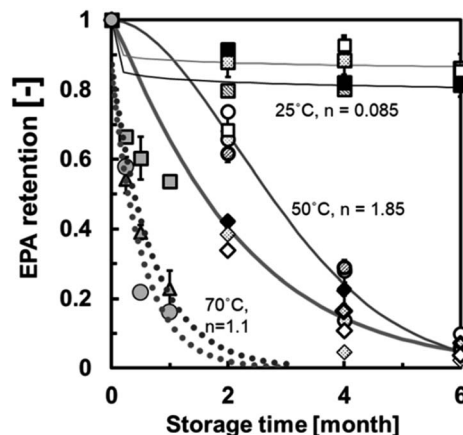


Fig. 14 EPA retention behaviour in spray-dried powder of emulsified krill oil at storage temperatures of 25, 50, and 70  $^{\circ}\text{C}$ . Grey lines represent the samples with a solid content of 50% that were subjected to mechanical homogenisation. Dot patterns represent the samples with a solid content of 50% that were subjected to high-pressure homogenisation. Symbols filled in black represent the samples with a solid content of 60% that were subjected to mechanical homogenisation. Symbols filled in white represent the samples with a solid content of 60% that were subjected to high-pressure homogenisation. Symbols filled in grey represent krill oil (Sultana *et al.*, 2021).<sup>47</sup> "Reproduced from ref. 47 with permission from Elsevier, copyright 2021".

activation for the rate constant of the change is expressed as a Gaussian distribution. Yoshii *et al.* (2001)<sup>50</sup> applied this Avrami equation to estimate the flavour release from spray-dried powder. Abd Ghani *et al.* (2017)<sup>51</sup> and Sultana *et al.* (2021)<sup>45</sup> also simulated the degradation kinetics of SQ and EPA and DHA encapsulated in spray-dried powder. The oxidation kinetics of PUFAs, such as EPA and DHA, are autocatalytic kinetics, as given in the above section. The oxidation or degradation kinetics of PUFAs in spray-dried powder may have a complex reaction mechanism with the antioxidant, barrier of the wall material, and emulsifier. As shown in Fig. 14, the

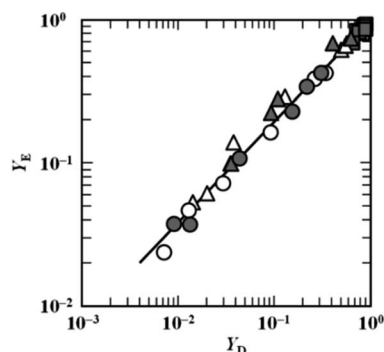


Fig. 15 Relationship between the fraction of unoxidised DHA ( $Y_D$ ) and unoxidised EPA ( $Y_E$ ) in microencapsulated Isada krill oil stored at ( $\square$ ) 25  $^{\circ}\text{C}$ , ( $\Delta$ ) 50  $^{\circ}\text{C}$  and ( $\circ$ ) 70  $^{\circ}\text{C}$ . The open and closed symbols indicate microcapsules with 50% and 60% solid content, respectively (Jimenez, Miyagawa, Yoshii, & Adachi, 2021).<sup>52</sup> "Reproduced from ref. 52 with permission from *J. Oreo Sci.*, copyright 2021".



Avrami equation is useful to correlate the PUFA retention in spray-dried powder using the corresponding mechanism number as the fitting parameter.

As mentioned above, the oxidation rate of a PUFA in a binary system was expressed using a kinetic model in which the rate was proportional to the product of the concentration of the unoxidised PUFA and the sum of the concentrations of the two oxidised PUFAs. The model was adopted to analyse the oxidation rates of **EPA** and **DHA** in microencapsulated Isada krill oil. Based on the model, the relationship between the fractions of unoxidised **EPA** ( $Y_E$ ) and unoxidised **DHA** ( $Y_D$ ) is expressed by the following equation (Jimenez *et al.*, 2021)<sup>52</sup>

$$Y_E = (Y_{E_0}/Y_{D_0}^\kappa)Y_D^\kappa \quad (18)$$

where  $Y_{E_0}$  and  $Y_{D_0}$  are the initial  $Y_E$  and  $Y_D$  values, respectively.  $\kappa = k_E/k_D$ , and  $k_E$  and  $k_D$  are the rate constants for the oxidation of **EPA** and **DHA**, respectively. Fig. 15 shows the relationship between  $Y_E$  and  $Y_D$  in the microencapsulated krill oil stored for six months at different temperatures. The plots lie on a straight line, indicating the suitability of eqn (18). The  $\kappa$  and  $Y_{E_0}/Y_{D_0}^\kappa$  values were 0.703 and 0.986, respectively. A value of  $\kappa$  less than 1 indicates that **DHA** is more easily oxidised than **EPA** (Jimenez *et al.*, 2021).<sup>52</sup>

## Conclusion

The stabilities of fish oil, krill oils and PUFA in spray-dried powder were affected by the morphology of spray-dried powder and the average oil-droplet and powder particle diameters. The ratio of the diameter of the oil droplet to the particle diameter was found to be an important factor in estimating the surface-oil ratio in the spray-dried powder. The stability of PUFAs in spray-dried powder is currently being investigated using a kinetic model with basic mass transfer phenomena and chemical reactions. The Avrami equation, which is compatible with various kinetic models, is useful to estimate the stability of fish oil and krill oil in the powder using the mechanism number and degradation or oxidation kinetic constant.

## Author contributions

Sultana wrote the encapsulation of fish oil and krill oil part. Adachi wrote and summarized the oil oxidation kinetics and all parts of this paper. Yoshii planned this review paper and summarized mainly the stability of fish and krill oils.

## Conflicts of interest

There are no conflicts to declare.

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